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PROGESTERONE UPREGULATES ACTIVITY AND PROTEIN EXPRESSION OF ECTO-5'-NUCLEOTIDASE IN ISCHEMIC BRAIN OF MALE WISTAR RATS

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ABSTRACT

Reduction of oxygen and glucose supply to the brain due to diminished cerebral blood flow leads to damage of tissue which in experimental conditions can be mimicked by permanent ligation of common carotid arteries (2VO). Besides numerous genomic and non-genomic processes, cerebral ischemia enhances expression of ecto-5'-nucleotidase (eN), a main enzyme in the central nervous system that produces potent neuromodulator and neuroprotector, adenosine. Since progesterone (P), a potent sex steroid, is recognized as neuroprotective, aim of this study was to examine whether repeated low-dose P treatment is capable to induce changes in activity and protein expression of eN, at rat cortical membrane fraction following 2VO. Obtained results indicate that P modulates investigated parameters and through stimulation of adenosine generation might promote cytoprotection in ischemic brain.

INTRODUCTION

The energy disturbance due to mild, permanent reduction of cerebral blood flow leads to the activation of microglia and astrocytes, subsequent production of inflammatory mediators, blood-brain barrier disruption and consequently, neuronal death [1]. This type of brain injury might induce massive release of ATP in the extracellular space, where it influences numerous aspects of neuronal, astrocytic, and microglial responses by activating purinergic receptors [2]. The extracellular ATP actions are controlled by members of ectonucleotidase family. Specifically, the rate-limiting enzyme in the ectonucleotidase pathway is ecto-5'-nucleotidase

(eN), which catalyzes the final step of AMP dephosphorylation and enables generation of adenosine, a potent neuromodulator and neuroprotector [3].

In the past few decades many therapeutic agents have been suggested for the treatment of ischemic brain injury. For instance, progesterone (P), a sex steroid, is involved in functions that extend beyond reproduction like synaptic plasticity, cognition, neurogenesis, etc. Although, its exogenous administration has been shown to improve the outcome in ischemic brain [4, 5], the mechanism by which P modulates adenosine generation, as one of the key neuroprotective agents, is not yet elucidated.

EXPERIMENTAL

Three months old male Wistar rats, kept according to the standards of Ethical Committee for the use of laboratory animals of University of Belgrade, VINCA Institute of Nuclear Sciences, Serbia, were subjected either to sham or 2VO surgery as previously described [4, 6]. Following 2VO surgeries animals were subcutaneously injected with P (4-Pregnene-3,20-dione, dissolved in commercial flax oil, 1.7 mg/ml, 2VO + P) or equal volume of vehicle (commercial flax oil, 2VO + V), while sham operated animals were subjected to vehicle (Sham + V). The treatments were administrated for seven consecutive days starting immediately after both types of surgeries [4].

To examine the activity and protein expression of eN, after decapitation and priory presented isolation of cortical synaptic plasma membranes (SPMs) [7], the samples were subjected to *in vitro* colorimetric assay for estimation of AMP hydrolysis and immunoblot analysis using anti CD73 antibody (Santa Cruz) for determination of changes in investigated enzyme's expression [7].

Presented results were obtained from three independent SPMs preparations and all measurements were done in triplicate. Statistical significance was determined by one-way ANOVA followed by Tuckey's posthoc test. Data are presented as mean ± SEM.

RESULTS AND DISCUSSION

Since our previous studies [4, 6] indicated that 2VO induces neurodegeneration due to provoked apoptotic processes observed in, both, cell body and terminals while P is capable to alter those processes by modifying BCl2 family expression and modulating caspase3 activity [4, 8], of interest was to examine whether repeated low-dose P treatment is able to modulate activity and protein expression of eN in the cerebral cortex.

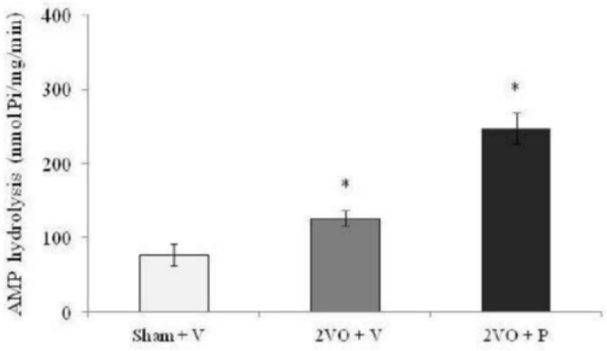

Figure 1. AMP hydrolysis at cortical SPMs of sham operated animals treated with vehicle (Sham + V) and 2VO rats subjected either to vehicle (2VO + V) or progesterone treatment (2VO + P) **as a marker of eN** activity presented as a mean ± SEM (* $p<0.05$).

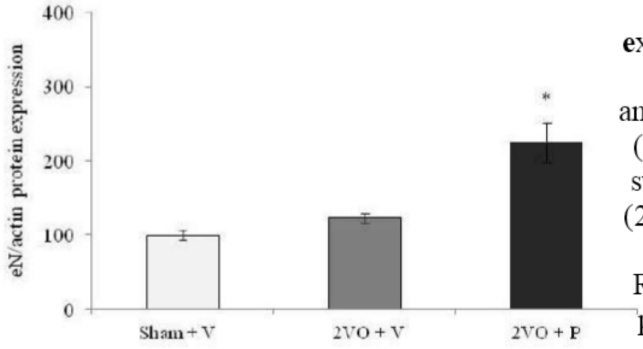

Figure 2. Protein expression of eN at cortical SPMs of sham operated animals treated with vehicle (Sham + V) and 2VO rats subjected either to vehicle (2VO + V) or progesterone treatment (2VO + P). Results are presented as a percentage of Sham + V, mean ± SEM (*$p<0.05$).

As shown in Figures 1. and 2, the rate of AMP hydrolysis in 2VO animals treated with vehicle increased significantly comparing to Sham + V group, but without altering eN protein abundance, while level of AMP hydrolysis and protein expression of eN were increased in 2VO animals treated with P.

The literature emphasizes that adenosine, a potent endogenous neuromodulator and homeostatic regulator, through balanced activation of its inhibitory and facilitatory receptors, mostly controls excitatory glutamatergic synapses thus mediating various neuroprotective events. In the brain that underwent ischemic conditions, observed augmentation in eN activity probably increased its concentration in extracellular milieu conferring its cytoprotection feature [9]. Thus, according to obtained results, it could be expected that in imposed experimental condition, P treatment might also lead to up-regulation of adenosine production in extracellular milieu attempting to ameliorate deleterious effects of 2VO.

CONCLUSION

According to presented results, repeated low-dose P treatment is able to modulate activity and protein expression of eN following 2VO surgery and thus, through increased adenosine production, moderates the effect of ischemic insult. Our findings should be considered significant for establishing an adequate therapy for ischemic brain injury, given much controversy remains in this area and the connection between ischemia, adenosine and sex steroids is still poorly understood.

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